# Post-harvest disinfestation of lightbrown apple moth, *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae), with an alkane

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**Abstract: Lightbrown apple moth (LBAM), *Epiphyas postvittana* Walker was used as a test insect to evaluate a post-harvest oil, C15 Ampol CPD, and a spray oil, C23 Ampol DC-Tron NR, both applied as dips. CPD was much more efficacious than C23 DC-Tron NR against exposed third-instar larvae. Higher oil concentrations were required to penetrate and kill larvae sheltering under the calyx of oranges. LBAM eggs were more susceptible to CPD oil than larval stages. LBAM larvae dipped in sub-lethal doses of oil continued to develop, but the fecundity of both males and females was reduced. DC-Tron had a significant effect on egglaying. CPD and C23 DC-Tron NR affected the fertility of eggs laid. CPD oil sprayed at 50 ml litre$^{-1}$ on adult LBAM moths reduced their fertility. Factors contributing to the higher efficacy of CPD and its potential use as a post-harvest treatment are discussed.**


**Keywords:** *Epiphyas postvittana*; disinfestation; petroleum; Tortricidae; post-harvest treatment

## 1 INTRODUCTION

The expanding international trade in fresh horticultural produce has increased the risk of the transport of insects on the surface of fruit. International quarantine regulations and inspectrion require fruit to be free of live pests. Lightbrown apple moth (LBAM), *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae), a leaf-roller pest of many horticultural crops in Australia and New Zealand, is a quarantine pest on exports of Australian navel oranges to the United States of America (USA). The calyx of an orange provides a protective shelter for a range of small arthropods. LBAM spins a hydrophobic silken domicile that enhances its survival during the normal washing process prior to packing. All fruit entering the USA must be free of LBAM and detection of live larvae presently results in fumigation with methyl bromide. Methyl bromide treatment reduces the quality of navel oranges and delays marketing. Fumigation has resulted in substantial financial losses to growers (Cain D, Chief Executive Officer Citrus Board of South Australia, private commun) and the use of methyl bromide will soon be restricted.[1] An alternative disinfestation treatment is needed to remove surface-dwelling pests from fruit.

We evaluated a C15 alkane as a post-harvest treatment against LBAM on citrus fruit. Our interest stemmed from historical use of petroleum oils for the control of arthropods[2–6] and a desire to use food-grade products in packing houses. We compared the relative efficacy of a petroleum spray oil and a light paraffinic oil against dipped *E postvittana* larvae and eggs. We also examined the effects of the alkane on adult fecundity because reduced fecundity has been reported on other pest species after use of petroleum oil.[7]

## 2 MATERIAL AND METHODS

### 2.1 *Epiphyas postvittana* colony

The LBAM used in the experiments were collected from a laboratory culture maintained since 1993, using the rearing procedure of Singh *et al.*[8]

### 2.2 Oil formulations

Caltex Australia Research and Development Laboratories, Brisbane Qld, supplied two oils, a homogeneous C15-alkane proposed for post-harvest dipping of citrus, C15 Ampol CPD, and a petroleum spray oil, C23 Ampol DC-Tron NR. The petroleum spray oil contains no alkanes and consists mostly of C15 mono-cyclic molecules with side chains. The C23 nomenclature refers to its mean equivalent *n*-paraffin carbon number; its 50% distillation temperature is the

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| Property | Test Method | C23 Ampol DC-Tron NR | C15 Ampol CPD |
|---|---|---|---|
| Distillation temperature at 101.33 kPa (°C) | ASTM[a] D-2887 | | |
| 10% point | | 346 | 253 |
| 50% point | | 385 | 275 |
| 90% point | | 411 | 304 |
| *n*-paraffin carbon number 50% point | HRGLC[b] | 23.0 | 15.2 |
| Mean relative molecular mass | ASTM D-4052 | 350 | 212 |
| Density at 15 °C ($g ml^{-1}$) | ASTM D-1298 | 0.8424 | 0.787 |
| Viscosity; Kinematic at 40.0 °C ($mm^2 s^{-1}$) | ASTM D-445 | 12.0 | 2.75 |
| Pour point (°C) | ASTM D-97 | −15 | +7 |
| Unsulfonated residue; (% minimum volume) | ASTM D-483 | 94 | >99 |
| Mean molecular volume 15 °C (ml $mole^{-1}$) | | 417 | 278 |
| Molecular types (%) | | | |
| Cp (paraffins) | | 70 | 100 |
| Cn (naphthenes) | | 28 | – |
| Ca (aromatics) | | 2 | – |

**Table 1.** Specifications of petroleum oils used in bioassays

[a] American Society for Testing and Materials.[10]
[b] Furness *et al.*[9]

same as the boiling point of a C23 alkane.[9] The specifications of both products are given in Table 1.

### 2.3 Acute mortality of non-domiciled cage-dipped larvae

Bioassays were performed using fully exposed larvae in wire cages to ensure good contact of oil emulsions with the larvae. In the field, LBAM larvae spin a hydrophobic silken domicile under the calyx of an orange. Larvae were selected after the desired developmental period (at 20 °C; 12 days for third instar, 21 days for fifth instar) and were treated with each product to determine their relative susceptibility. For dose-mortality bioassays, rates were selected to give a range of mortalities dependent on product and instar used. The rates selected for DC-Tron NR against third instars were 1.0, 2.5, 5.0, 10 and 25 ml $litre^{-1}$. For the alkane, CPD, rates were 0.25, 0.5, 0.75, 1.0 and 2.5 ml $litre^{-1}$ and 0.5, 1.0, 2.5, 5.0 and 10.0 ml $litre^{-1}$ for third and fifth instars, respectively. The controls were dipped in water only and the experiment was replicated four times.

For each dipping, larvae were collected from media pots using fine camel-hair brushes and transferred in groups of 10 into 400 mm diameter fine wire mesh spherical cages. The caged larvae were suspended in a 500-ml beaker of well-agitated emulsion for 30 s. After treatment, the larvae were removed and placed into rearing media within a plastic container. The containers were sealed and placed in a rearing room (20(±2) °C, natural lighting) and mortality assessed at 4 h and finally at 24 h. Larvae were counted as dead if they did not move after repeated prodding with a needle. After 24 h, all larvae were placed in 80% aqueous ethanol for 12–24 h. The head-capsule widths of 15 randomly selected larvae from each treatment were measured, using a stereomicroscope with an eyepiece graticule, to confirm their developmental stage.

### 2.4 Acute mortality of domicile-protected larvae under sepals

Fresh pesticide- and insect-free navel oranges with intact calyxes were used to determine the effects of D-C-Tron NR and CPD on domicile-protected larvae under calyxes. After harvest, the fruit were stored at 5 °C before use. At least 100 fruit were used in each experiment. Before infestation, each calyx was cleaned to remove debris, and the sepals gently lifted. Two larvae were then placed on opposite sides of each calyx. The sepals were then placed back in position and their edges covered with a fine film of macerated medium prepared according to Singh *et al.*[8] Third instars were used because preliminary work showed that they were the largest stage that could be successfully placed in pairs under the sepals using soft-nosed forceps. A 15-mm diameter plastic phial was then inverted over each calyx. A rubber band was used to hold the phial in position. Establishment of the larvae under the calyxes was determined at 24 and 48 h. Larvae were considered established if webbing could be seen.

The effect of the oils on larval mortality was determined by dipping groups of 10 infested oranges in a 500-ml beaker of well-agitated emulsion for 30 s. Infested fruit bioassay rates were 1.0, 2.5, 5.0, 10, 25 and 50 ml $litre^{-1}$. The controls were dipped in water only and the experiment was replicated four times. After treatment, the infested fruit were placed on a shallow colander to drain for 5 min. Larvae were stored

and assessed in the same manner as described for caged larvae. However, any larva found dead immediately after treatment was discarded on the assumption that it had died prior to dipping.

### 2.5 Acute mortality of dipped eggs

DC-Tron NR, with a relative molecular mass of 350, is sufficiently non-volatile to be an effective ovicide.[11] Petroleum oils with similar physical characteristics to DC-Tron NR are known to be effective against the eggs of tortricid moths.[11–13] Bioassays to determine the ovicidal effectiveness of CPD, with a relative molecular mass of 212, are described in this section.

Egg masses were obtained by placing unmated pairs of adults in plastic cups (Polar Cup, Adelaide) in a naturally lit rearing room at 20(±3) °C and 55 (±5)% RH. Two pairs were placed in each cup. Single cotton wool wicks soaked in 10% honey were placed in each cup. Females began laying eggs on the sides of the cups after 24 h. After 72 h, sturdy scissors were used to cut plastic discs from the cups with individual egg masses attached to them. Only egg masses with 30–60 eggs were used for bioassays: small egg masses and large egg masses with many eggs laid over each other were discarded. Two- (green), seven- (yellow) and 10- (black-head) day-old eggs were used for the bioassays.

In an initial experiment, 10-day-old egg masses were dipped individually in 1 ml litre$^{-1}$ emulsions of either C23 DC-Tron NR, CPD or water for 30 s. Each treatment was replicated six times. After dipping, each disc was placed upright against the inner edge of a Petri dish lined with filter paper to allow excess solution to drain. Each dish was placed in a naturally lit rearing room at 20(±3) °C and 55 (±5)% RH. Egg development and hatch was checked every two days until all control eggs had hatched or desiccated. Subsequently, the initial experiment was repeated using CPD at 0.05, 0.1, 0.5, 1.0 and 5 ml litre$^{-1}$ emulsions. Dose-responses were derived for each of the selected stages of egg development.

### 2.6 Chronic larval mortality

Caged groups of 15 third-instar larvae were dipped, according to the procedures described previously, in separate 0.1 ml litre$^{-1}$ emulsions of each oil, a dose predetermined to give low acute mortality after 24 h. Control larvae were dipped in water. After 24 h, larval activity was scored according to criteria described by Firko and Hayes.[14] Any larva dead or unable to right itself easily was discarded. The 10 most active larvae in each treatment replicate were placed in larval rearing media in plastic trays and reared in a naturally lit room at 20(±2) °C. Mortality was assessed every four to seven days until all larvae had either died or emerged from pupae as adults.

### 2.7 Fecundity and fertility after larval exposure to sub-lethal doses

Caged groups of 10 third-instar larvae were dipped in separate 0.1 ml litre$^{-1}$ emulsions of oils or in water using the same procedures used to determine chronic larval mortality. The sexes were separated as pupae. After eclosion, single unmated pairs of adults were placed in plastic cups, provided with 10% honey (as described previously), and held at 20(±3) °C and 55 (±5)% RH under natural lighting. The numbers of eggs laid per female and percentage hatch were assessed in all treatments after all eggs in the control had hatched or desiccated.

### 2.8 Fecundity and fertility of sprayed adults

Groups of 40 newly eclosed moths of each sex were anaesthetised with carbon dioxide, placed on filter-paper-lined Petri dishes and sprayed with either water or 10, 25 or 50 ml CPD litre$^{-1}$ emulsions using a Potter spray tower (Burkhard, UK). Each spray comprised 4 ml solution applied at 103 kPa with a 15-s settling time. After treatment, moths of the same sex were placed in small plastic cages and held under natural light at 20(±3) °C and 55 (±5)% RH. Mortality was assessed after 24 h. Moths treated with 50 ml litre$^{-1}$ CPD were paired in three combinations and placed in separate plastic cups: control male with control female, control male with CPD female, and CPD male with control female. There were five pairs per treatment. A 10% honey solution was provided as food and the pairs were held in a rearing room at 20(±3) °C and 55 (±5)% RH under natural lighting for 72 h. The number of eggs laid per female and percentage hatch were assessed in all treatments after all eggs in the control had hatched or desiccated.

### 2.9 Effects of oil-contaminated food on larval feeding and development

Normal rearing medium (Singh *et al*[8]) was mixed with CPD at a rate of 10 ml litre$^{-1}$ during the last stages of preparation. Approximately 30 g of mixed medium was then placed in 6-cm-diameter pots, allowed to solidify, and then stored at 5 °C for seven days. Tests with Automate Dye Red B (Petrafin, Sydney) indicated that the mixing procedures resulted in even distribution of the oil in the medium. On day 7 groups of 10 third-instar larvae were weighed in Petri dishes and placed in the media pots. The larvae were then reared at 20 (±3) °C and 55 (±5)% RH under natural light. Each media pot and group of larvae was weighed after 3, 7 and 15 days when pupation and adult eclosion rates were determined. Each of the two treatments comprised four replicates.

### 2.10 Data analysis

Dose-mortality data were transformed to probits and analysed using POLO-PC[15] to calculate LC values, dose-response lines and likelihood ratio tests to compare responses between different treatments.

Statistix 4.1[16] was used for analysis of variance (ANOVA). Bartlett's test was used to test the hypothesis of equal variances. If the hypothesis was rejected, a square root transformation of the data was used before analysis of variance. Percentage data were

**Table 2.** Dose-mortality response of *Epiphyas postvittana* larvae to dipping in C23 Ampol DC-Tron NR and C15 Ampol CPD

| Oil type | Instar (situation) | Sample (n) | Slope (± SEM) | $LC_{50}$ (ml litre$^{-1}$) (95% CL) | $LC_{90}$ (ml litre$^{-1}$) (95% CL) | $\chi^2$ |
|---|---|---|---|---|---|---|
| CPD | III (caged) | 240 | 4.10 (±0.55) | 0.47 (0.36–0.58) | 0.97 (0.76–1.57) | 39.15 |
| | V (caged) | 207 | 2.98 (±0.37) | 3.27 (2.64–3.94) | 11.68 (8.92–17.5) | 0.33 |
| | III (under calyx) | 309 | 3.01 (±0.33) | 3.31 (2.69–3.92) | 8.80 (7.26–11.33) | 3.12 |
| DC-Tron | III (caged) | 240 | 1.33 (±0.23) | 12.41 (8.45–22.28) | 113.9 (49.6–618.4) | 18.78 |

arcsine square root transformed before analysis. The formula used was as follows:

$$\text{T value} = 180/\pi \times (\arcsin(\text{sqrt}(\text{value}/100)))$$

Mean separation was determined using the least significant difference method.

## 3 RESULTS

### 3.1 Acute mortality of dipped LBAM larvae

Non-domicile cage-dipped LBAM larvae were >100 times more susceptible to CPD than to C23 DC-Tron NR (Table 2) at the $LC_{90}$ estimates. Comparison of $LC_{90}$ estimates indicated that fifth-instar larvae were almost 12 times more tolerant to CPD than third instars. The regressions for the two oils against third-instar larvae were significantly different ($\chi^2 = 180$, df = 2: $P < 0.001$) and not parallel ($\chi^2 = 25.97$, df = 1: $P < 0.001$) (Fig 1).

Results with artificially infested fruit indicated that domicile-protected third-instar larvae were >8 times less susceptible to CPD than non-domicile protected third instars (Table 2). The $LC_{99}$ for the protected larvae was 19.55 ml litre$^{-1}$ (95% CL; 14.55–30.01).

### 3.2 Acute mortality of dipped eggs

High mortality occurred in LBAM eggs dipped in 1 ml litre$^{-1}$ oil emulsions compared to controls, with mortality levels of 5.43%, 99.29% and 89.85% for controls, CPD and DC-Tron NR, respectively ($F = 100.57$, df = 3, $P < 0.001$).

LBAM eggs showed marked differences in susceptibility at different ages when dipped in CDP oil. All egg ages showed increased mortality at higher concentrations (oil rates: F = 38.1, df = 2, $P < 0.001$). Two- and 10-day-old eggs were equally and significantly more susceptible than seven-day old eggs when dipped in 0.1 or 1 ml litre$^{-1}$ CPD emulsions (egg age: F = 22.9, df = 2, $P < 0.001$). However, differences in susceptibility due to egg age were not constant, with 100% mortality in all egg ages at 5 ml litre$^{-1}$ emulsions (oil rate by egg age interaction: F = 4.61, df = 4, $P < 0.005$) (Table 3).

### 3.3 Chronic larval mortality

There were no significant effects on mortality or development of non-domicile-protected third-instar larvae cage-dipped in 0.1 ml emulsions of either CPD or C23 DC-Tron NR (Table 4). More than 80% of larvae in all treatments developed to normal adults.

### 3.4 Fecundity and fertility after larval exposure to sub-lethal doses

Females that emerged from C23 DC-Tron NR-treated larvae laid fewer eggs than females reared from water or CPD-treated larvae. Females from the former treatment also laid fewer eggs per egg mass than those from the other two treatments. Hatch of eggs from both oil treatments was significantly lower than hatch of eggs in the control, but the two oil treatments did

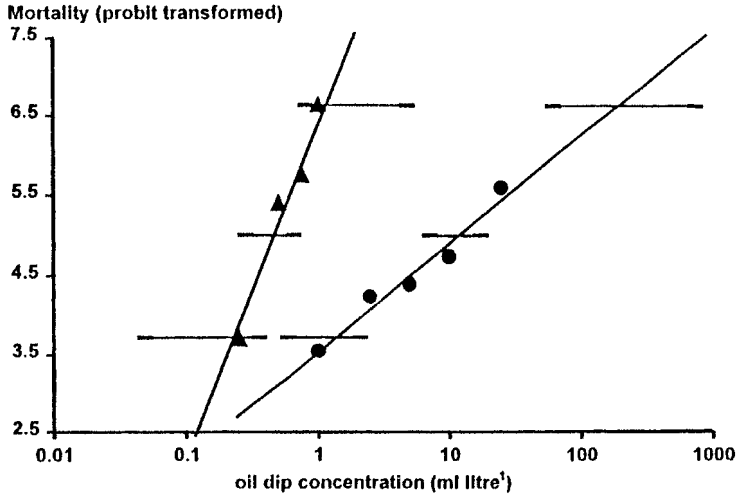


**Figure 1.** Dose-response regression and selected 95% fiducial limits for third-instar *Epiphyas postvittana* larvae to post-harvest oil dip, (▲) C15 Ampol CPD and (●) C23 Ampol DC-Tron NR, after 24 h exposure.

| Rate (ml litre$^{-1}$) | Egg age (days) | Total eggs[a] | Eggs unhatched | Mortality (%) (±SEM) |
|---|---|---|---|---|
| 0 | 2 | 253 | 34 | 13.4 (±5.8) |
| | 7 | 299 | 27 | 9.0 (±3.2) |
| | 10 | 248 | 29 | 11.7 (±2.7) |
| 0.1 | 2 | 264 | 175 | 66.3 (±16.1) |
| | 7 | 312 | 17 | 5.4 (±2.2) |
| | 10 | 263 | 135 | 51.3 (±19.4) |
| 1.0 | 2 | 265 | 265 | 100.0 (±0.0) |
| | 7 | 301 | 73 | 24.3 (±14.7) |
| | 10 | 274 | 253 | 92.3 (±1.2) |
| 5.0 | 2 | 248 | 248 | 100.0 (±0.0) |
| | 7 | 306 | 306 | 100.0 (±0.0) |
| | 10 | 316 | 316 | 100.0 (±0.0) |

[a] Values are the sum of six egg masses.

**Table 3.** Mortality of three developmental stages of *Epiphyas postvittana* eggs dipped in C15 Ampol CPD oil formulation

not differ significantly from each other. The results were quite variable (Table 5).

### 3.5 Fecundity and fertility of sprayed adults

No mortality occurred in either sex 24 h after spraying with 10, 25 and 50 ml litre$^{-1}$ CPD (Table 6). Comparison of water-sprayed pairs with water-sprayed adults mated with a CPD-oil-sprayed adult of the opposite sex showed no significant difference in the number of eggs per female or the number of egg masses per female (Table 6). However, the fertility of eggs laid by females sprayed with CPD and mated with water-sprayed males, and water-sprayed females mated with CPD-sprayed males, was significantly lower than the fertility of eggs laid by control pairs (Table 6). Fertility was affected regardless of the sex of the oil-treated adult in the pair.

### 3.6 Effects of oil-contaminated food on larval feeding and development

Exposure of larvae to medium mixed with a 10 ml litre$^{-1}$ dose of CPD oil did not affect their mortality after 15 days; the average mortality in the CPD treatment was 17.5% compared to 12.5% in the control (the critical value for the comparison was 13.2; $P < 0.05$). However, larvae reared on CPD-treated medium developed more slowly. After 15 days, pupation in the oil treatment and in the control were 35% and 67.5% respectively (the critical value for comparison was 16.9: $P < 0.01$). Larvae reared on the oil-treated medium were significantly lighter throughout their development than those in the controls (Table 7). Larval weight gain was significantly higher for control larvae on day 3 ($F = 38.1$, $df = 1$, $P < 0.001$), day 7 ($F = 13.4$, $df = 1$, $P < 0.05$) and day 15 ($F = 9.1$, $df = 1$, $P < 0.05$). Larvae exposed to oil diet consistently consumed less food than control larvae, with significant differences in the amount of food consumed on day 3 ($F = 24.8$, $df = 1$, $P < 0.01$), day 7 ($F = 37.6$, $df = 1$, $P < 0.001$) and day 15 ($F = 383$, $df = 1$, $P < 0.001$). Pupae that developed from the larvae in the oil treatment were also significantly lighter than pupae from the controls (mean pupal weight: CPD = 10.52 mg; Control = 23.12 mg; critical value 4.63; $P < 0.001$).

## 4 DISCUSSION

### 4.1 Acute mortality of oil dips

CPD was much more efficacious in dips against LBAM larvae than DC-Tron NR. These products are quite different and their acute effects on the larvae possibly relate to distinct physical characteristics owing to their different molecular volumes. The former is a homogeneous C15 alkane with a relative molecular mass of 212. It is liquid at room temperature (alkanes with $\geq$16 carbon atoms are waxes at room temperature), and has a molecular volume of 278 ml mole$^{-1}$ at 15 °C. The latter contains no alkanes and its C23 nomenclature refers to its mean equivalent *n*-paraffin carbon number; its 50% distillation temperature is the same as the boiling point of a C23 alkane.[9] It has an average relative molecular mass of 350 and most molecules are paraffinic (%$C_P \geq 60\%$) C15 mono-cyclic molecules with side chains. Their average molecular volume is 417 ml mole$^{-1}$ at 15 °C.

Interestingly, the higher efficacy of the C15 alkane is contrary to the results of field trials with petroleum spray oils where the lowest molecular mass fractions showed the poorest efficacy.[4–6,17,18] Other factors must be contributing to the efficacy of the alkane in

| Formulation | Mortality (±SEM)[a] | Emergence (±SEM)[a] |
|---|---|---|
| Control | 0.25 (±0.25)a | 9.25 (±0.25)a |
| CPD | 1.00 (±0.41)a | 8.50 (±0.65)a |
| DC-Tron | 1.50 (±0.29)a | 8.25 (±0.25)a |

[a] Mean of four replicates of 10 larvae. Means within a column followed by the same letter are not significantly different according to one-way analysis of variance ($P > 0.05$, least significant difference).

**Table 4.** Post-24-h mortality and development of third-instar *Epiphyas postvittana* larvae dipped at 0.1 ml litre$^{-1}$ in C23 Ampol DC-Tron or C15 Ampol CPD, and held at 20 °C for 14 days

| Formulation | No of eggs/♀[a] (± SEM) | No of eggs/mass[a] (± SEM) | Eggs hatched[b] (%) (± SEM) |
|---|---|---|---|
| Control | 184.8 (±70.1)a | 37.1 (±7.57)a | 78.9 (±19.7)a |
| CPD | 190.8 (±69.9)a | 14.6 (±5.10)a | 23.1 (±23.1)b |
| DC-Tron | 7.2 (±4.53)b | 2.1 (±1.35)b | 2.4 (±2.43)b |

[a] Means of at least five replicates. Means within a column followed by the same letter are not significantly different according to one-way analysis of variance of the data ($P > 0.05$, least significant difference).

[b] Means within a column followed by the same letter are not significantly different according to one-way analysis of variance of arcsin square root-transformed percentage data ($P > 0.05$, least significant difference).

**Table 5.** Fecundity and fertility of female *Epiphyas postvittana* adults dipped as third-instar larvae in 0.1 ml litre$^{-1}$ of C23 Ampol DC-Tron or C15 Ampol CPD oil formulations

our tests. The application technique of dipping rather than spraying, the oil-depositing characteristics of the formulations[19] and/or an alternative mode of action may all influence the relative efficacy of the oils. Different emulsifiers are used in each product, which may have had some influence on the results. The effects of emulsifiers on plant cuticles and waxes has been well described and reviewed.[20] However, although the effects of emulsifiers on plant and arthropod cuticles are probably similar[21] the different emulsifiers used in the two products are unlikely to have been primarily responsible for the effects of the latter on LBAM larvae.

LBAM eggs were more susceptible to CPD than the larval stages. We showed that CPD in post-harvest dips acts as an effective ovicide at $\geq$5 ml litre$^{-1}$. The C15 alkane probably penetrates more rapidly than DC-Tron NR, but may dissipate before it can interrupt gas exchange. Further studies are required to determine the similarity between its mode of action and reported effects of other oils on gas exchange through egg membranes,[12] and interference with hormones and enzymes after they penetrate the chorion.[22] The results also showed that susceptibility to CPD varied with the age of eggs kept at 20 °C; two- and 10-day-old eggs were more sensitive than seven-day-old eggs. In comparison, the eggs of oriental fruit moth, *Grapholita molesta* (Busck), and codling moth, *Cydia pomonella* L, become less susceptible to petroleum spray oils as they develop.[12,13]

### 4.2 Chronic effects of oils

Chronic effects of the larval dips on the fecundity and fertility of adult females varied. DC-Tron NR significantly reduced fecundity whereas CPD produced similar egg numbers to controls. Both oils significantly reduced egg hatch. The rate of dissipation of DC-Tron NR from tissue is probably slower than that of the C15 alkane and this may have contributed to its increased effects. Petroleum oils with molecular mass equivalents of <C19 are considered unsuitable for field application due to a lack of persistence.[23] The dipping process offers increased access into the tracheal system of insects where oil may persist longer or affect different physiological processes. However, we do not know why the oils would lead to chronic effects and it would clearly be of interest to determine them. Ebeling[7] observed the effects of petroleum spray oils on the fecundity of red scale, *Aonidiella aurantii* (Maskell), treated as adults but did not propose a mode of action.

Although CPD sprays did not affect adult LBAM mortality, the oil did reduce fertility. However, this was applied at above the field rates used for petroleum spray oils (generally $\leq$2% v/v) and given costs and other factors associated with practical control would not be cost-effective as a control measure. Riehl *et al*[13] found that, although petroleum spray oils did not kill adult codling moth when applied topically, they did affect their fertility. The mechanism is unknown, but in our study both sexes were affected. As such, oil is likely to be disrupting general physiological processes, rather than giving rise to a highly specific event.

Inclusion of CPD in rearing medium affected LBAM larvae. Larvae developed more slowly and were lighter than normal larvae. This may have been partially due to feeding avoidance because larvae consumed less oil-treated medium than larvae reared on normal medium. Baxendale and Johnson[24] observed anti-feedant effects of petroleum oils on

| Treated pairs | No of eggs/♀ [a] (± SEM) | No of egg masses/♀ [a] (± SEM) | Eggs hatched[b] (%) (± SEM) |
|---|---|---|---|
| Control ♂ ,Control ♀ | 277.4 (±0.5)a | 11.8 (±0.97)a | 66.3 (±17.3)a |
| CPD ♂,Control ♀ | 147.2 (±52.3)a | 14.6 (±2.82)a | 17.4 (±17.4)b |
| Control ♂,CPD ♀ | 147.2 (±54.5)a | 14.2 (±1.74)a | 10.6 (±10.6)b |

[a] Means of at least five replicates. Means within a column followed by the same letter are not significantly different according to one-way analysis of variance of the data ($P > 0.05$, least significant difference).

[b] Means within a column followed by the same letter are not significantly different according to one way analysis of variance of arcsin square root-transformed percentage data ($P > 0.05$, least significant difference).

**Table 6.** Fecundity and fertility of adult *Epiphyas postvittana* sprayed with 50 ml litre$^{-1}$ of C15 Ampol CPD oil

**Table 7.** Cumulative weight gain and food consumption of *Epiphyas postvittana* larvae after exposure to 10 ml litre$^{-1}$ Ampol CPD oil incorporated into medium, and held at 20°C

| Treatment | | Days of exposure | | |
|---|---|---|---|---|
| | | 3 | 7 | 15 |
| Avge weight gain per larva[a] (mg) (±SEM) | Control | 7.4 (±0.67) | 25.8 (±1.76) | 50.1 (±5.24) |
| | CPD | 3.6 (±0.20) | 17.9 (±1.24) | 43.2 (±1.73) |
| Avge food consumed per larva[a] (mg) (±SEM) | Control | 32.1 (±17.3) | 97.3 (±35.6) | 203.2 (±16.0) |
| | CPD | 21.4 (±12.7) | 71.9 (±21.2) | 142.0 (±26.8) |

[a] Values are means of four replicates.

euonymous webworm, *Yponomeuta multipunctella*, but recorded no subsequent effect on development.

The oils may solubilise lipids in the insect cuticle and cell membranes. Oils have been demonstrated to disrupt plant cellular membranes.[25] Generally, oils high in unsaturated hydrocarbons have been implicated in 'corroding' tissues.[2,26] However, saturated oils, such as the C15 alkane used in this study, may still cause disruption of cell tissues, but at a much slower rate. Oranges dipped in CPD oil recorded higher levels of ethylene production (Mark Hodgkinson, unpublished) consistent with increased cell disruption.[27]

### 4.3 Development of oils as post-harvest dips

Citrus packing houses are designed to process large volumes of fruit in a short period. A disinfestation treatment applied in a packing house would need to penetrate beneath the calyx very quickly, typically 30 s or less. Any product used should leave no harmful residues and be compatible with current machinery. In this study, CPD is considered suitable because it is food grade and its low viscosity can aid in penetration beneath the calyx. Insect spiracles are quickly penetrated when immersed in low-molecular-mass oil fractions.[2]

In Australia, citrus packing houses dip oranges on delivery in large dipping tanks filled with a fungicide solution. Our work shows C15 alkanes to be highly efficacious when applied in dips. They are more efficacious than petroleum spray oil, DC-Tron NR, applied in the same manner. Oil rates would need to be significantly higher to control larvae sheltering on fruit compared to exposed larvae. This is an important consideration in the determination of an effective rate in packing houses where whole fruit are immersed. Our results indicate that CPD would be effective as a postharvest treatment for LBAM larvae if used at 30 ml litre$^{-1}$. At this concentration, 99% (upper 95% CL) of third-instar larvae established under the calyx were killed. Larger larvae would be exposed and thus die at this rate. Any eggs present would also be killed. Surviving larvae would be of reduced thrift, and any adult moths that emerged would be likely to be infertile.

Commercial trials have shown CPD to be effective in the post-harvest control of mites and mealybug on citrus.[28] Alkanes applied in the packing house offer a simple technology to treat fruit where experience has indicated pest problems at destination. Currently, internal and surface pests are fumigated with methyl bromide. Alkanes may be able to replace the use of methyl bromide as a commodity treatment on 'hitch-hiker' pests with a relatively low initial incidence. An alkane would most likely be used as a component of a systems approach[29] to meet quarantine security.

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## REFERENCES

1 Anonymous, Copenhagen amendment of the Montreal protocol on substances that deplete the ozone layer (1992).
2 de Ong ER, Technical aspects of petroleum oils and oil sprays. *J Econ Ent*, **19**:733–745 (1926).
3 Chapman PJ, Avens AW and Pearce GW, San Jose scale control experiments. *J Econ Ent*, **37**:305–306 (1944).
4 Riehl LA and Carman GE, Narrow cut petroleum fractions of naphthenic and paraffinic composition for control of California red scale. *J Econ Ent*, **46**:1007–1113 (1953).
5 Riehl LA and Jeppson LR, Narrow cut petroleum fractions of naphthenic and paraffinic composition for control of citrus red mite and citrus bud mite. *J Econ Ent*, **46**:1014–1020 (1953).
6 Trammel K, Properties of petroleum oils in relation to citrus red mite eggs. *J Econ Ent*, **58**:595–601 (1965).
7 Ebeling W, Effect of oil on California red scale at various stages of development. *Hilgardia* **10**:95–125 (1936).
8 Singh P, Clare GK and Ashby MD, *Epiphyas postvittana*, in *Handbook of Rearing Insects II*. ed by Singh P and Moore RF, Elsevier, New York, pp 271–283 (1985).
9 Furness GO, Walker DA, Johnson PG and Riehl LA, High resolution GLC specifications for plant spray oils. *Pestic Sci* **18**:113–128 (1986).
10 American Society for Testing and Materials, *Standards for petroleum products and lubricants*, ASTM, Philadelphia PA (1998).
11 Fiori BJ, Smith EH and Chapman PJ, Some factors influencing the ovicidal effectiveness of saturated petroleum oils and synthetic isoparafins. *J Econ Ent* **56**:885–888 (1963).
12 Smith EH and Pearce GW, The mode of action of petroleum oils as ovicides. *J Econ Ent*, **25**:173–180 (1948).
13 Riehl H, Halaj J, Kreowski WB, Hilton RJ and Westigard PH, Laboratory evaluation of mineral oils for control of codling moth (Lepidoptera: Tortricidae). *J Econ Ent*, **88**:140–147 (1995).

14 Firko MJ and Hayes JL, Quantification of larval resistance to cypermethrin in tobacco budworm (Lepidoptera:Noctuidae) and the effects of larval weight. *J Econ Ent*, **83**:140–146 (1990).
15 LeOra Software, *POLO-PC: a user's guide to Probit or Logit analysis*, LeOra Software, Berkeley, CA (1987).
16 Analytical Software, *Statistix® version 4.1: users manual*, Analytical Software, Tallahassee, FL (1994).
17 Chapman PJ, Pearce GW and Avens AW, Relation of composition to the efficiency of foliage or summer type petroleum fractions. *J. Econ. Ent*, **36**:241–247 (1943).
18 Chapman PJ, Lienk SE, Avens AW and White RW, Selection of a plant spray oil combining full pesticide efficiency with minimum plant injury hazards. *J. Econ Ent*, **55**:737–744 (1962).
19 Campbell MM, Efficiency of summer spray oils in relation to the concentration of the emulsifying agent. *Exp Rec Dept Agr S Aust*, **7**:36–42 (1972).
20 Gaskin RE, Phytotoxicity of agrochemical surfactants, *Proc 4th Internat. Symp on Adjuvants for Agrochemicals*, NZ FRI Bulletin no 193. pp 193–200 (1995).
21 Hurst H, Insect cuticle as an asymmetrical membrane. *Nature (London)*, **147**:388–389 (1941).
22 Hoskins WM, Recent contributions of insect physiology to insect toxicology and control. *Hilgardia*, **13**:307–386 (1943).
23 Ebeling W, *Subtropical Entomology*, Lithotype Process Co, San Francisco. pp 173–179 (1950).
24 Baxendale RW and Johnson WT, Evaluation of summer spray oil on amenity plants. *J Arboriculture*, **14**:220–225 (1988).
25 van Overbeek J and Blondeau R, Mode of action of phytotoxic oils. *Weeds*, **3**:55–65 (1954).
26 Brown AWA, *Insect control by Chemicals*, John Wiley & Sons, New York. 69 pp (1951).
27 Knocke M and Noga GJ, Effect of non-ionic surfactants on ethylene release and leaf growth of *Phaseolus vulgaris*. *Sci Horticulturae*, **46**:1–11 (1991).
28 Taverner PD and Bailey PT, Commercial trial of citrus postharvest oil for removal of surface pests, *SARDI Research Report Series No 5*, South Australian Research and Development Institute, Adelaide, Aust (1995).
29 Hata TY, Hara AH, Hu BKS, Kaneko RT and Tenbrink VL, Pest management before harvest and insecticidal dip after harvest as a systems approach to quarantine security of red ginger. *J Econ Ent*, **85**:2310–2316 (1992).